D1230–D1234 *Nucleic Acids Research, 2012, Vol. 40, Database issue* *Published online 10 November 2011*
doi:10.1093/nar/gkr806

A database of immunoglobulins with integrated tools: DIGIT

Anna Chailyan[1], **Anna Tramontano**[1,2,*] **and Paolo Marcatili**[1*]

[1]Department of Physics, Sapienza University of Rome, P.le A. Moro, 5-00185, Italy and [2]Center for Life Nano Science, Sapienza, Istituto Italiano di Tecnologia,Viale Regina Elena, 291-00161, Rome (I), Italy

Received August 14, 2011; Accepted September 13, 2011

ABSTRACT

The DIGIT (Database of ImmunoGlobulins with Integrated Tools) database (http://biocomputing.it/digit) is an integrated resource storing sequences of annotated immunoglobulin variable domains and enriched with tools for searching and analyzing them. The annotations in the database include information on the type of antigen, the respective germline sequences and on pairing information between light and heavy chains. Other annotations, such as the identification of the complementarity determining regions, assignment of their structural class and identification of mutations with respect to the germline, are computed on the fly and can also be obtained for user-submitted sequences. The system allows customized BLAST searches and automatic building of 3D models of the domains to be performed.

INTRODUCTION

Successful recognition of foreign antigens by antibodies (or immunoglobulins) is crucial for the defense of an organism against pathogens and strictly depends upon the enormous diversity of the sequences and structures of these molecules. At the same time, these molecules play an exceptionally important role in diagnosis, therapy and biotechnology applications.

The effective usage of antibodies in all these applications demands knowledge and understanding of their sequence and structural properties in order to study the molecular basis of their specificity, their ‘evolutionary’ history within the organism and to be able to modify them as in humanization experiments or in the design of combinatorial libraries.

There are several resources aimed at providing an integrated view of the sequences and structures of antibodies, each with advantages and disadvantages. The most renowned one is the Kabat database (1), which has been the textbook (and originally was indeed released as such) for immunologists. Unfortunately, this is now only available at a cost and is not regularly updated. The Abysis portal (2) provides some of the needed services, such as the possibility of querying the database by accession number, antigen, author name, reference, year of first publication, chain type (lambda or heavy or both), species, etc., but is limited to amino acid sequences only and cannot be used for nucleotide sequences. The Vbase2 database (3) is limited to human and mouse germline sequences and, most importantly, has not been updated since 2006. IMGT (4) is a database of fully annotated sequences of immunoglobulins and T-cell receptors from human and other vertebrates (150 species). It does not provide sequence-searching tools for amino acid sequences nor it includes information on light and heavy-chain pairing of the entries.

To overcome some of the shortcomings of the systems described above and the problems that we ourselves faced when analyzing real life cases (5–9), we took advantage of our long-lasting experience in immunoglobulin sequence and structure analysis and structural prediction (8,10–19) and developed the DIGIT (Database of ImmunoGlobulins with Integrated Tools) system.

The annotations in our database include information on the type of antigen, the respective germline sequences and on pairing information between light and heavy chains.

The user can query the database using the antigen type, source organism, accession number, chain type (heavy, lambda and kappa) or free text (disease, process, etc.) with the option of selecting only complete immunoglobulins (i.e. cases where both the correctly paired light and heavy-chain sequences are available).

Other annotations are computed on the fly (and therefore can also be obtained for user-submitted sequences), for example:

(1) numbering of the sequence according to the Kabat–Chothia numbering scheme (20);

*To whom correspondence should be addressed. Tel: +39 06 49914550; Fax: +39 06 4440062; Email: anna.tramontano@uniroma1.it
Correspondence may also be addressed to P. Marcatili. Tel: +39 06 49914550; Fax: +39 06 4957697; E-mail: paolo.marcatili@uniroma1.it

(2) identifications of the complementarity determining regions (CDRs) in the sequence and of the framework regions;
(3) assignment of the canonical structures for the CDRs (21);
(4) identification of mutations with respect to the germline;
(5) automatic link to our 3D modeling tool for immunoglobulin variable domains (14); and
(6) sequence searching that, given the input immunoglobulin sequence of interest (amino acid or nucleotide sequence of heavy-chain variable domain sequence; light-chain variable domain sequence or both), retrieves the closest sequences (sorted according to the *E*-value or percentage of sequence identity).

We believe that this is a much-needed resource as the information that it contains is either absent from any other database or can only be obtained by browsing several sites, most of which is not regularly updated and we are convinced that DIGIT will be extremely useful to researchers interested in immunology as well as to scientists performing experiments such as antibody humanization, stabilization and functionalization.

IMMUNOGLOBULIN VARIABLE DOMAIN STRUCTURE AND NOMENCLATURE

Immunoglobulins are glycoproteins specifically binding to one or a few closely related antigens. All immunoglobulins have a four-chain structure as their basic unit. They are composed of two identical light chains (L) and two identical heavy chains (H) held together by inter-chain disulfide bonds and by non-covalent interactions. Two domains, a variable and a constant one, form the light chain, while one variable domain and three constant domains usually form the heavy chain. Most of the diversity of the variable domains resides in three regions from each chain, called the hypervariable or CDRs. These are named according to the chain they belong to and the order they appear in the sequence (L1, L2, L3, H1, H2 and H3). The regions between the CDRs in the variable region are called the framework regions (FW). Immunoglobulin light chains are classified as kappa or lambda according to their serological and sequence properties.

Immunoglobulin sequences are usually numbered according to a common scheme (Kabat–Chothia) aimed at assigning the same number to topologically equivalent residues (20). This is a widely adopted standard for numbering the residues of antibodies in a consistent manner.

The relationship between the amino acid sequences of immunoglobulins and the 3D structures of their antigen binding sites has been extensively studied leading to the identification of relatively few residues that, through their packing, hydrogen bonding or the ability to assume unusual main chain conformations, are primarily responsible for the main-chain conformations of the hypervariable regions (17,18,21). The commonly occurring main-chain conformations of the hypervariable regions are called 'canonical structures'. The canonical structure definitions can be effectively used to predict the structure of immunoglobulin variable domains (12,14).

DIGIT SYSTEM OVERVIEW

DIGIT consists of four main modules:

(1) the DB search tool allows the user to retrieve entries by chain type (heavy, lambda and kappa), source organism, antigen, NCBI accession number and free text search;
(2) the Browse tool can be used for inspecting the content of the database;
(3) the Sequence Search tool permits to perform BLAST searches of the database using a user provided nucleotide or amino acid sequence of the light or heavy chain or both as queries; and
(4) the analysis toolbox provides, for a given immunoglobulin sequence:
    (a) the Kabat–Chothia numbering of the chain;
    (b) the canonical structures of each of the CDRs;
    (c) the mutations with respect to the corresponding germline sequence; and
    (d) the construction of a 3D model of the molecule through our modeling PIGS (Prediction of Immunoglobulin Structure) server (14) when both light and heavy chains have been retrieved or uploaded for an entry.

There are provisions for saving and later retrieving the results as well as for filtering the output files according to user-specified keywords.

The database is regularly updated every 90 days.

An overview of the system is schematically shown in Figure 1.

DATA SOURCES

The database presently contains 145 759 heavy-chain sequences and 71 404 light chain sequences (47 168 kappa type and 24 236 lambda type) retrieved using isotype-specific HMM profiles developed by us, with assigned canonical structures for the hypervariable loops and data on the type of antigen as well as the pairing information of immunoglobulin heavy and light chains (9672 total pairs).

Sequences were retrieved from the NCBI database (22) (June 22, 2011) using as query (immunoglobulin OR immunoglobulins OR antibodies OR antibody OR IG OR Ab OR heavy OR light OR Fab OR FV).

Light and heavy-chain sequences as well as the type of light chain (lambda or kappa) were identified by comparing them with HMMs developed on purpose (available from the DIGIT web site).

Pairing between light and heavy chains, an information not reported in sequence databases, was obtained by identifying heavy and light-chain sequences reported by the same author and referring to the same publication either if the latter only contained one pair of light and heavy-chain sequences or if the NCBI description field for both

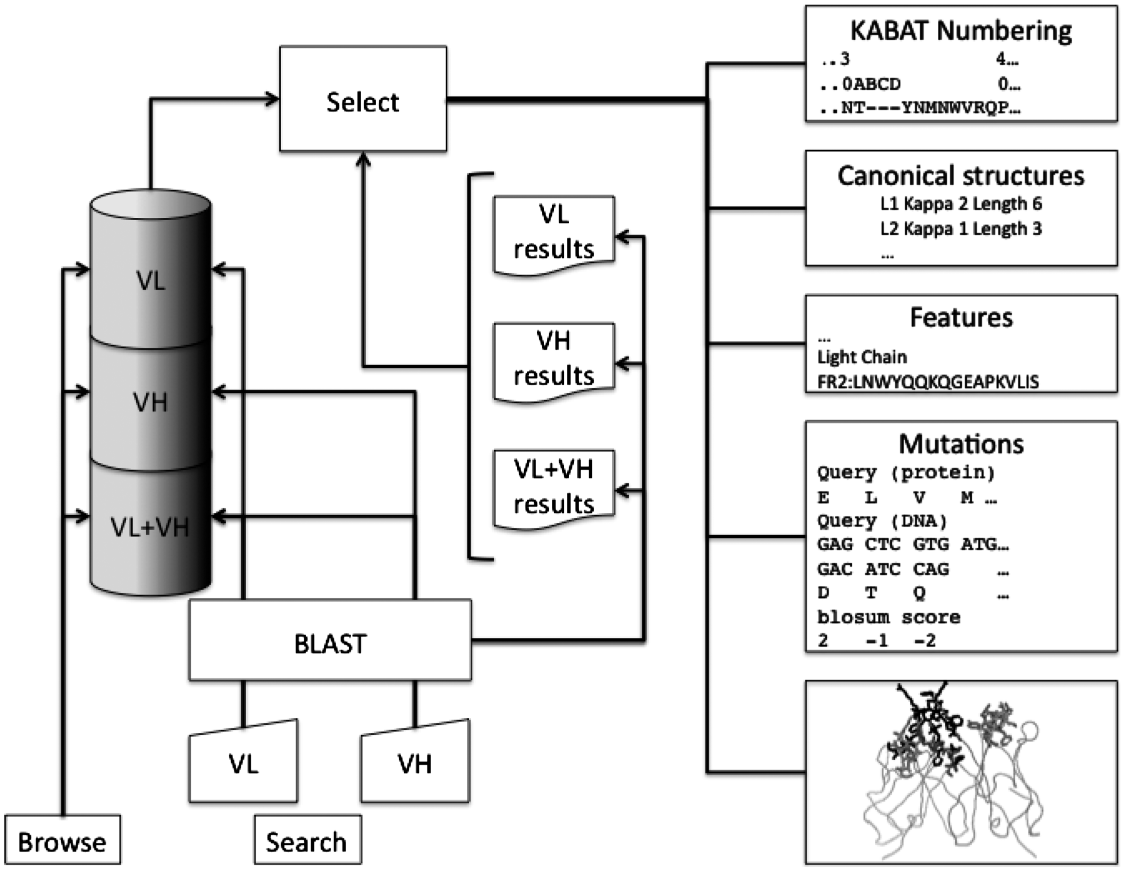


Figure 1. Schematic view of the options provided by DIGIT. After selecting an entry obtained by either browsing or searching the database or directly submitted, the user can retrieve the Kabat–Chothia numbering of the sequence, the canonical structures of the CDRs, the mutations with respect to the germline and the 3D model of the molecule. Note that the input can be either a nucleotide or an amino acid sequence. The system provides the possibility of printing and saving the results as well as of aligning the sequences of the displayed entries.

chains reported an unambiguous identifier after the keywords 'clone', 'sample' or 'isolate'.

The name of the antigen was retrieved by searching in the NCBI description field the words following the 'anti' term. The type of antigen was attributed using a vocabulary developed on purpose.

A manual analysis of the automatic assignment on a few hundreds immunoglobulins stored in the database showed that only a handful of assignments were incorrect, and these were mainly due to ambiguous description fields in the NCBI entry.

DB SEARCH TOOL

The database can be queried by chain type (heavy, lambda, kappa), source organism, antigen, accession number or through a free text search.

The output of the DB search operation includes the corresponding NCBI identifier, a description of the antigen, the organism source and the reference to the original article.

Fields are linked to the corresponding NCBI entry, to the PUBMED record for the article and to a summary page reporting the genomic locus, the NCBI definition of the entry, the organism, the reference to the original article, the sequences of the light and heavy chain, the isotype of the lambda chain (kappa or lambda), the Kabat–Chothia numbering and, if available, the antigen and the antigen type (protein, peptide, carbohydrate, hapten and small molecule).

A toggle button can be used to select an entry for further analysis (see 'Analysis Tools' section).

BROWSE TOOL

This is a simple interface for browsing the content of the database and selecting an entry to analyze. The user can select the chain type or (optionally) the progressive number of the entry from which to start. The output page contains a list of the first 25 retrieved entries and of buttons to move to the next or previous 25 entries.

SEQUENCE SEARCH TOOL

The user can provide the amino acid or nucleotide sequence(s) of a light or heavy chain or both.

The system performs one or more Blast searches with default parameters. If the input consists of a light and heavy chain, the database is searched with the light chain, with the heavy chain and with both. The latter result is useful, for example, for humanization experiments or for selecting a modeling template.

In both cases, for example, it might be convenient to select the chains of the same immunoglobulin as templates for both the light and heavy chain rather than chains coming from different antibodies, since even minor differences in the interface can lead to a different packing geometry and affect the topology of the antigen binding site (11).

In the description below, we will assume that the user wishes to search the database with a complete variable domain including both the light and heavy chains. The only difference from the case when only one of the two chains is used as input is that, obviously, only the results for the selected chain are available in this case.

ANALYSIS TOOLS

Upon completion of the search, the user can access several pages using tabs labeled 'Blast L', 'Blast H', 'Blast L+H', 'Kabat numbering', 'Canonical structures', 'Features', 'Mutations' and '3D model' (Figure 2).

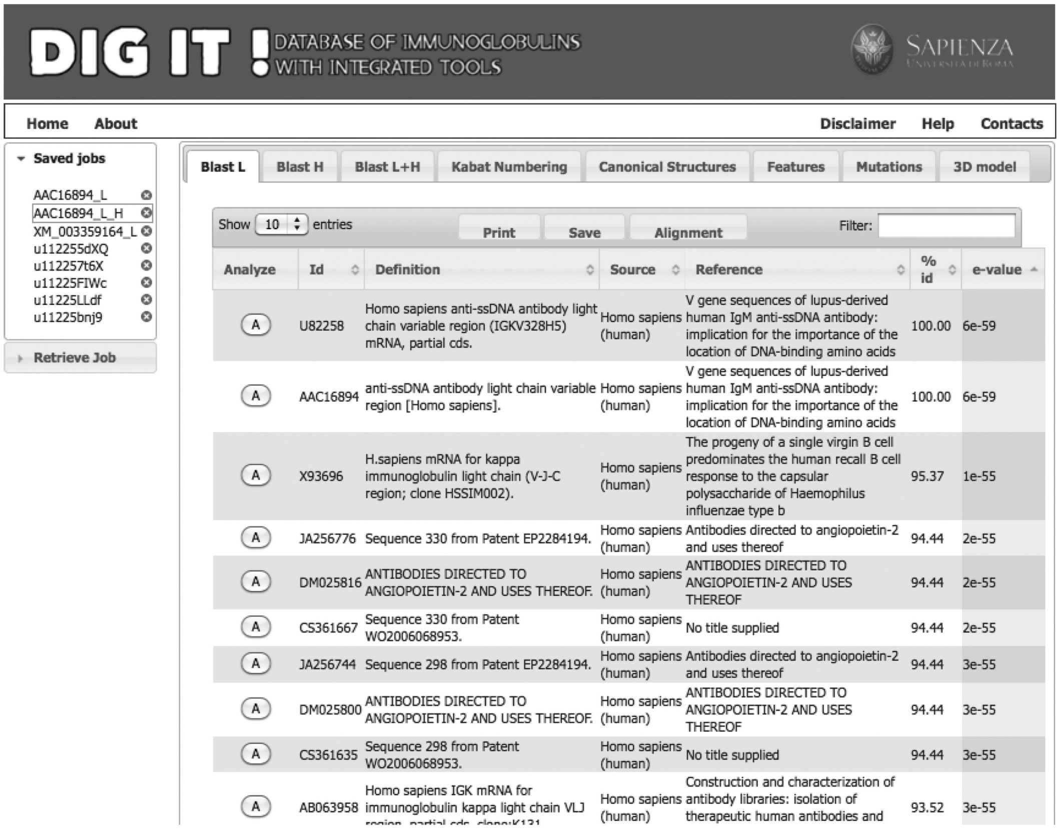
The first three options provide the Blast results for the light and heavy chain and those obtained by concatenating the two chains and searching a dataset where paired light and heavy-chain sequences are concatenated as well.

These pages contain the NCBI sequence identifier of the retrieved sequence(s), the definition(s), the reference, the antigen, the percentage of sequence identity and the *E*-value as reported by BLAST. Each column can be used for sorting the results and columns can be moved around using the mouse.

The Kabat numbering page reports the alignment of the input sequence with the commonly used Kabat–Chothia numbering scheme (20). The canonical structures (describing the main chain conformation of the CDRs) are also reported together with the length of the CDRs.

The Features page is a summary reporting the sequences of the various regions of the molecule separately in the order they appear in the sequence (Framework 1, CDR1, Framework 2, CDR2, Framework 3, CDR3 and Framework 4).

The 'Mutations' page includes information about the mutations with respect to the germline of the selected sequence. If the input sequence was provided as a nucleotide sequence, both the nucleotide and the corresponding amino acid mutations are shown. The Blosum62 score for the amino acid variation is also shown below each mutation.



Analyze	Id	Definition	Source	Reference	% id	e-value
A	U82258	Homo sapiens anti-ssDNA antibody light chain variable region (IGKV328H5) mRNA, partial cds.	Homo sapiens (human)	V gene sequences of lupus-derived human IgM anti-ssDNA antibody: implication for the importance of the location of DNA-binding amino acids	100.00	6e-59
A	AAC16894	anti-ssDNA antibody light chain variable region [Homo sapiens].	Homo sapiens (human)	V gene sequences of lupus-derived human IgM anti-ssDNA antibody: implication for the importance of the location of DNA-binding amino acids	100.00	6e-59
A	X93696	H.sapiens mRNA for kappa immunoglobulin light chain (V-J-C region; clone HSSIM002).	Homo sapiens (human)	The progeny of a single virgin B cell predominates the human recall B cell response to the capsular polysaccharide of Haemophilus influenzae type b	95.37	1e-55
A	JA256776	Sequence 330 from Patent EP2284194.	Homo sapiens (human)	Antibodies directed to angiopoietin-2 and uses thereof	94.44	2e-55
A	DM025816	ANTIBODIES DIRECTED TO ANGIOPOIETIN-2 AND USES THEREOF.	Homo sapiens (human)	ANTIBODIES DIRECTED TO ANGIOPOIETIN-2 AND USES THEREOF	94.44	2e-55
A	CS361667	Sequence 330 from Patent WO2006068953.	Homo sapiens (human)	No title supplied	94.44	2e-55
A	JA256744	Sequence 298 from Patent EP2284194.	Homo sapiens (human)	Antibodies directed to angiopoietin-2 and uses thereof	94.44	3e-55
A	DM025800	ANTIBODIES DIRECTED TO ANGIOPOIETIN-2 AND USES THEREOF.	Homo sapiens (human)	ANTIBODIES DIRECTED TO ANGIOPOIETIN-2 AND USES THEREOF	94.44	3e-55
A	CS361635	Sequence 298 from Patent WO2006068953.	Homo sapiens (human)	No title supplied	94.44	3e-55
A	AB063958	Homo sapiens IGK mRNA for immunoglobulin kappa light chain VLJ region, partial cds, clone:K131	Homo sapiens (human)	Construction and characterization of antibody libraries: isolation of therapeutic human antibodies and	93.52	3e-55

Figure 2. The output page of the DB search tool. The left panel lists previous jobs the results of which can be retrieved. The current job is enclosed in a box. The various tabs provide access to the corresponding results. Each of the entries can be used as starting point for a new analysis by clicking the 'A' button. The results can be printed, saved or filtered according to user-defined keywords. The multiple sequence alignment for all the displayed entries, obtained by clicking the 'Alignment' button, is displayed on a new page.

Finally, the user can directly obtain a 3D model of the input sequence through the PIGS immunoglobulin modeling tool (14).

In all cases when a list of sequences is displayed on the page, it is possible to obtain an alignment of their sequences in a new window. If both light and heavy chains are listed on the page (as for example in a Blast 'L+H' output), the alignment will include both light and heavy-chain sequences separated by the '//' symbol.

CONCLUSIONS

Several biomedical and biotechnological projects need to take advantage of a detailed understanding of the immunoglobulin features. Humanization and combinatorial library design require an analysis of the properties of the framework of the molecules and of their similarity with other antibodies from the same or a different organism, immunologically based diagnosis can be helped by comparing the sequences of different immunoglobulins as well as by understanding their mutation patterns with respect to the germline and by inspecting their known or predicted 3D structures.

In all these cases, the light and heavy chains of the antibody cannot be treated as separate entities (as is the case in sequence databases). Furthermore, the analyses have to be easy to perform within a single site and reported in a language and with an organization reflecting the flowchart of the design of an experiment.

We believe that the DIGIT system described here meets all these requirements and can become a 'one stop shop' for the biomedical and biotechnological community interested in immunoglobulins.

FUNDING

King Abdullah University of Science and Technology (KAUST) (Award number KUK-I1-012-43); FIRB Proteomica, Ministery of Health grant (Contract No. Onc_Ord 25/07); IIT SEED project. Funding for open access charge: King Abdullah University of Science and Technology (KAUST) (Award number KUK-I1-012-43).

REFERENCES

1. Johnson,G. and Wu,T.T. (2001) Kabat Database and its applications: future directions. *Nucleic Acids Res.*, **29**, 205–206.
2. Martin,C.R. (2010) *Protein Sequence and Structure Analysis of Antibody Variable Domains*. Springer, New York.
3. Retter,I., Althaus,H.H., Munch,R. and Muller,W. (2005) VBASE2, an integrative V gene database. *Nucleic Acids Res.*, **33**, D671–674.
4. Giudicelli,V., Duroux,P., Ginestoux,C., Folch,G., Jabado-Michaloud,J., Chaume,D. and Lefranc,M.P. (2006) IMGT/LIGM-DB, the IMGT comprehensive database of immunoglobulin and T cell receptor nucleotide sequences. *Nucleic Acids Res.*, **34**, D781–784.
5. Donini,M., Morea,V., Desiderio,A., Pashkoulov,D., Villani,M.E., Tramontano,A. and Benvenuto,E. (2003) Engineering stable cytoplasmic intrabodies with designed specificity. *J. Mol. Biol.*, **330**, 323–332.
6. Ghiotto,F., Fais,F., Valetto,A., Albesiano,E., Hashimoto,S., Dono,M., Ikematsu,H., Allen,S.L., Kolitz,J., Rai,K.R. *et al.* (2004) Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. *J. Clin. Invest.*, **113**, 1008–1016.
7. Ghiotto,F., Marcatili,P., Tenca,C., Calevo,M.G., Yan,X.J., Albesiano,E., Bagnara,D., Colombo,M., Cutrona,G., Chu,C.C. *et al.* (2011) Mutation pattern of paired immunoglobulin heavy and light variable domains in chronic lymphocytic leukemia B-cells. *Mol. Med.*, in press; doi:10.2119/molmed.2011.00104.
8. Sollazzo,M., Castiglia,D., Billetta,R., Tramontano,A. and Zanetti,M. (1990) Structural definition by antibody engineering of an idiotypic determinant. *Protein Eng.*, **3**, 531–539.
9. Zibellini,S., Capello,D., Forconi,F., Marcatili,P., Rossi,D., Rattotti,S., Franceschetti,S., Sozzi,E., Cencini,E., Marasca,R. *et al.* (2011) Stereotyped patterns of B-cell receptor in splenic marginal zone lymphoma. *Haematologica*, **95**, 1792–1796.
10. Chailyan,A., Marcatili,P., Cirillo,D. and Tramontano,A. (2010) Structural repertoire of immunoglobulin lambda light chains. *Proteins*, **79**, 1513–1524.
11. Chailyan,A., Marcatili,P. and Tramontano,A. (2010) The association of heavy and light chain variable domains in antibodies: implications for antigen specificity. *FEBS J.*, **278**, 2858–2866.
12. Chothia,C., Lesk,A.M., Tramontano,A., Levitt,M., Smith-Gill,S.J., Air,G., Sheriff,S., Padlan,E.A., Davies,D., Tulip,W.R. *et al.* (1989) Conformations of immunoglobulin hypervariable regions. *Nature*, **342**, 877–883.
13. Helmer-Citterich,M., Rovida,E., Luzzago,A. and Tramontano,A. (1995) Modelling antibody-antigen interactions: ferritin as a case study. *Mol. Immunol.*, **32**, 1001–1010.
14. Marcatili,P., Rosi,A. and Tramontano,A. (2008) PIGS: automatic prediction of antibody structures. *Bioinformatics*, **24**, 1953–1954.
15. Morea,V., Lesk,A.M. and Tramontano,A. (2000) Antibody modeling: implications for engineering and design. *Methods*, **20**, 267–279.
16. Morea,V., Tramontano,A., Rustici,M., Chothia,C. and Lesk,A.M. (1997) Antibody structure, prediction and redesign. *Biophys Chem.*, **68**, 9–16.
17. Morea,V., Tramontano,A., Rustici,M., Chothia,C. and Lesk,A.M. (1998) Conformations of the third hypervariable region in the VH domain of immunoglobulins. *J. Mol. Biol.*, **275**, 269–294.
18. Tramontano,A., Chothia,C. and Lesk,A.M. (1990) Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins. *J. Mol. Biol.*, **215**, 175–182.
19. Tramontano,A. and Lesk,A.M. (1992) Common features of the conformations of antigen-binding loops in immunoglobulins and application to modeling loop conformations. *Proteins*, **13**, 231–245.
20. Al-Lazikani,B., Lesk,A.M. and Chothia,C. (1997) Standard conformations for the canonical structures of immunoglobulins. *J. Mol. Biol.*, **273**, 927–948.
21. Chothia,C. and Lesk,A.M. (1987) Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol.*, **196**, 901–917.
22. Benson,D.A., Karsch-Mizrachi,I., Lipman,D.J., Ostell,J. and Sayers,E.W. (2009) GenBank. *Nucleic Acids Res.*, **37**, D26–D31.